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Original articles

The −48 C/T polymorphism in the presenilin 1 promoter is associated with an increased risk of developing Alzheimer's disease and an increased Aβ load in brain

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Revised version received
29 March 2001
Accepted for publication
3 April 2001

Abstract

Mutations in the presenilin 1 gene (*PS1*) account for the majority of early onset, familial, autosomal dominant forms of Alzheimer's disease (AD), whereas its role in other late onset forms of AD remains unclear. A −48 C/T polymorphism in the *PS1* promoter has been associated with an increased genetic risk in early onset complex AD and moreover has been shown to influence the expression of the *PS1* gene. This raises the possibility that previous conflicting findings from association studies with homozygosity for the *PS1* intron 8 polymorphism might be the result of linkage disequilibrium with the −48 CC genotype. Here we provide further evidence of increased risk of AD associated with homozygosity for the −48 CC genotype (odds ratio=1.6). We also report a phenotypic correlation with $A\beta_{40}$, $A\beta_{42(43)}$, and total Aβ load in AD brains. The −48 CC genotype was associated with 47% greater total Aβ load (p<0.003) compared to CT + TT genotype bearers. These results suggest that the −48 C/T polymorphism in the *PS1* promoter may increase the risk of AD, perhaps by altering *PS1* gene expression and thereby influencing Aβ load.
(*J Med Genet* 2001;38:353–355)

Keywords: Alzheimer; presenilin; promoter; polymorphism

Epidemiological and molecular studies suggest that multiple genes and environmental factors underlie the aetiology of AD. To date, four genes have been shown to play a role in AD.[1] The apolipoprotein E (*APOE*) gene is recognised as a major risk factor for complex forms of AD (that is, non mendelian patterns of inheritance), while pathogenic mutations in the amyloid precursor protein (*APP*), presenilin 1 (*PS1*), and presenilin 2 (*PS2*) genes are responsible for some rare, early onset, autosomal dominant forms, with 18-50% of cases being caused by mutations in the *PS1* gene.[2] In vitro and in vivo studies show that pathogenic mutations in *PS1* favour Aβ peptide production, particularly $A\beta_{42(43)}$,[3] the species suspected to initiate the formation of amyloid plaques.[4] This observation is of particular relevance given the greater $A\beta_{42(43)}$ deposition in the brains of patients bearing *PS1* mutations,[5] greater than found in complex forms of AD.

The role of genetic variations of *PS1* in complex forms of AD is unclear. Despite our original observation of an association between a single nucleotide polymorphism (SNP) in intron 8 of *PS1* and AD,[6] these data have not always been replicated and no functional role has been ascribed to this polymorphism.[7] It has been suggested that the association with the intron 8 polymorphism might be spurious or that the disease associated allele might be in linkage disequilibrium with a functional variant elsewhere in the gene. The −48 C/T polymorphism in the *PS1* promoter is a possible candidate and has recently been associated with an increased risk of early onset AD (EOAD).[8 9] We therefore tested the impact of this polymorphism in our UK white AD cases and controls and have hypothesised that because the *PS1* pathogenic mutations affect APP metabolism, this polymorphism might also modulate Aβ load in human AD brains.

Table 1 Allele and genotype distribution of the −48 C/T polymorphism

	No	Allele distribution (%)		Genotype distribution (%)		
		C	T	CC	CT	TT
Manchester population						
AD cases	123	233 (0.95)	13 (0.05)	110 (0.89)	13 (0.11)	—
Control	117	213 (0.91)	21 (0.09)	97 (0.83)	19 (0.16)	1 (0.01)
Scottish population						
AD cases	164	301 (0.92)	27 (0.08)	137 (0.84)	27 (0.16)	—
Control	365	649 (0.89)	81 (0.11)	290 (0.80)	69 (0.19)	6 (0.02)
Total population						
AD cases	287	534 (0.93)*	84 (0.07)	247 (0.86)†	40 (0.14)	—
Control	482	862 (0.89)	102 (0.11)	387 (0.80)	88 (0.18)	7 (0.02)
Dutch population[12]						
AD cases	96	183 (0.95)‡	9 (0.05)	88 (0.92)‡	7 (0.07)	1 (0.01)
Control	117	209 (0.89)	25 (0.11)	94 (0.80)	21 (0.18)	2 (0.02)

*p<0.01, †p<0.03, ‡p<0.04.
Number of subjects (frequency). No differences were detected in allele and genotype frequencies between UK centres. All genotype distributions were in Hardy-Weinberg equilibrium.

Methods

STUDY POPULATION

All AD cases were white (n=287, mean age=67.1 (SD 13.0) years, mean age at onset=63.1 (SD 10.4) years, 49% males), ascertained from two UK centres, the central belt of Scotland (n=164, 25% had been

Table 2 Allele and genotype distribution of the −48 C/T polymorphism in early and late onset populations

		Allele distribution (%)		Genotype distribution (%)		
	No	C	T	CC	CT	TT
Early onset						
AD cases	176	334 (0.93)	24 (0.07)	152 (0.86)	24 (0.14)	—
Control	254	454 (0.89)	54 (0.11)	202 (0.79)	50 (0.20)	2 (0.01)
Late onset						
AD cases	105	196 (0.93)	14 (0.07)	91 (0.87)	14 (0.13)	—
Control	209	374 (0.90)	44 (0.10)	170 (0.81)	34 (0.17)	5 (0.02)

Number of subjects (frequency).

Table 3 $A\beta_{40}$, $A\beta_{42(43)}$, and total Aβ loads according to the -48 CT genotype in AD brains

	CC n=81	CT n=17	TT n=1	p
$A\beta_{40}$	4.2 (4.0)	1.9 (1.9)	0.2	<0.02
$A\beta_{42(43)}$	10.5 (4.2)	8.3 (5.5)	8.6	<0.04
Total Aβ	14.7 (6.7)	10.2 (6.8)	8.8	<0.01

Values are % area occupied in Brodmann area 8/9 of the frontal cortex (mean (SD)).

confirmed as definite AD; in this definite AD population, only one case had early onset AD) and Greater Manchester (n=123, all of which were probable AD cases). Diagnoses of definite or probable AD were established according to DSM-III-R and NINDCS-ADRDA criteria. Early (EOAD) and late onset AD (LOAD) were defined as cases with onset before 65 years of age or ≥65 years of age (EOAD n=177, LOAD n=110). The proportion of AD cases with a family history was 20%. The white controls were collected from the same geographical areas as the AD patients and were defined as subjects without DSM-III-R dementia criteria and with full integrity of their cognitive functions (n=482, mean age=62.5 (SD 14.4) years, 42% males). Ethical approval for the study and informed consent was obtained from all participants and their relatives and data were anonymised to ensure subject confidentiality.

BRAIN SAMPLES

Brains from a further 99 cases of definite AD (mean age at onset=65.8 (SD 10.0) years, mean age at death=74.3 (SD 9.1) years, 49% males) were collected from the Greater Manchester area. DNA was extracted from the frozen brain tissues of these cases by standard methods. The proportion of tissue area occupied by $A\beta_{40}$, $A\beta_{42(43)}$, and total Aβ ($A\beta_{40}$+$A\beta_{42(43)}$) was quantified in immunohistochemically stained sections from Brodmann areas 8/9 of the frontal cortex, as previously reported.[4]

GENOTYPING

APOE and −48 C/T genotypes were determined as previously reported.[9 10] The −48 TT and CT genotypes were replicated to confirm the complete digestion of the C allele fragment.

Table 4 $A\beta_{40}$, $A\beta_{42(43)}$, and total Aβ loads according to the −48 C/T polymorphism and APOE genotypes in AD brains

	Non ε4 bearers			ε4 bearers		
	CC n=26	CT+TT n=5	p	CC n=54	CT+TT n=11	p
$A\beta_{40}$	2.5 (2.4)	1.0 (0.9)	<0.09	5.1 (4.3)	2.4 (2.1)	<0.05
$A\beta_{42(43)}$	10.7 (4.6)	6.9 (1.2)	<0.07	10.4 (4.1)	9.7 (6.3)	<0.23
Total Aβ	13.1 (6.2)	7.9 (1.6)	<0.06	15.5 (7.0)	12.2 (7.6)	<0.11

Values are % area occupied in regions 8 and 9 (mean (SD)).

STATISTICAL ANALYSIS

Univariate analyses were performed by Pearson's χ^2 test. In the multivariate analysis, we tested the hypothesis that possession of the −48 CC genotype increases the risk of AD (that is, −48 CC versus −48 CT + TT genotypes).[8 9] The effect of the CC variant on risk for AD was assessed using a multiple logistic regression model adjusted for age and gender. The amyloid load for −48 CC bearers was compared with −48 CT + TT bearers using the Wilcoxon non-parametric test.

Results

The distributions of the −48 C/T alleles and genotypes for AD and control subjects are shown in table 1. The frequency of the −48 T allele in the control population was 11%, similar to that previously reported in a Dutch population.[9] We observed a significant difference for both allele and genotype distributions between the AD and control populations (p=0.01 and p=0.03, respectively), the −48 CC genotype being associated with an increased risk of developing AD (OR=1.55, 95% CI 1.03-2.35, p=0.04). Similar trends were observed in the Scottish and Manchester populations (respectively, OR=1.31, 95% CI 0.81-2.13, p=0.27 and OR=1.75, 95% CI 0.82-3.69, p=0.14) (table 1). The effect of this polymorphism was similar whether familial or sporadic disease (in the population without a family history, OR=1.63, 95% CI 1.04-2.57, p=0.034) or whether definite or probable AD cases were analysed separately. This effect appears to be independent of the *APOE* ε4 allele (OR=1.68, 95% CI 1.09-2.59, p=0.02, adjusted for the presence of at least one ε4 allele). No significant interaction with *PS1* was detected with age or sex. We also observed a stronger effect of −48 CC genotype in EOAD cases compared to LOAD and age matched control cases; however, they were not significantly different (OR=1.56, 95% CI 0.91-2.75, p=0.11 and 1.27, 95% CI 0.72-2.82, p=0.31, respectively) (table 2).

We next tested the hypothesis that the −48 C/T polymorphism may be associated with Aβ peptide load in brains from AD patients. We observed that all three measures of Aβ load ($A\beta_{40}$, $A\beta_{42(43)}$, and total Aβ) were significantly increased in −48 CC bearers (table 3). Subjects bearing the −48 CC genotype presented a 100% and 37% increase in $A\beta_{40}$ (p=0.007) and $A\beta_{42(43)}$ (p=0.01) load, respectively, leading to a 47% (p=0.003) increase in total Aβ load. This increased load appears to be independent of the *APOE* ε4 allele (table 4). We found no relationship between −48 CC genotype and the age of onset of disease in the Manchester cohort of brains, but we did detect a trend towards a shorter duration of illness (CC=7.8 (SD 3.7) years, CT and TT=9.5 (SD 3.1) years, p=0.067 with Wilcoxon non-parametric test).

Discussion

In this study we provide further evidence of an association in our UK population between AD and the −48 C/T polymorphism in the *PS1* gene promoter. We detected an overall in-

creased frequency of the −48 CC genotype in AD cases (OR=1.6) whereas a slightly stronger effect had been reported in a Dutch EOAD sample (OR=2.6).[9] We also found a trend towards a stronger effect in EOAD cases in our population. Interestingly, in a second Dutch LOAD cohort, no association with the −48 C/T polymorphism was detected.[10] Thus, we could speculate that the −48 C/T polymorphism may have a greater impact in earlier onset forms of the disease, as do the dominant AD mutations in the *PS1* gene.

Considerable research effort has been directed towards understanding the function of PS1 protein. Evidence suggests that it plays a role in cell trafficking[11] and apoptosis[12] and chromosomal segregation.[13] PS1 has been implicated in the γ-secretase activity that generates the carboxy-terminus of Aβ peptides.[14] Because mutations in *PS1* in familial AD are directly implicated in APP metabolism and production of Aβ, we hypothesised that variations of *PS1* expression because of polymorphisms in the promoter may similarly influence the production of Aβ. Indeed, Aβ peptide production can be reduced after inhibition of PS1 expression in cultured cells.[15] In this present study, we have reported that the −48 CC genotype correlates with increased Aβ deposition, supporting the view that genetic variants in the *PS1* promoter increase the risk of developing AD by modulating PS1 expression and consequently APP metabolism. Mutations in the *PS1* gene increase the total amount of Aβ secreted and deposited through selectively influencing the activity of γ-secretase in favour of the production of $A\beta_{42(43)}$.[16] We have shown here that the −48 C/T polymorphism is associated with brain increases in both $A\beta_{40}$ and $A\beta_{42(43)}$, implying that its modulatory effect on PS1 activity is unselective, at least as far as the composition of the C-terminal Aβ peptides that are produced is concerned. Hence, while the −48 C/T polymorphism leads to increased deposition of Aβ, this might be achieved by driving more APP per se through the catabolic cascade rather than, as is the case with the *PS1* mutants, by facilitating the preferential production of the more highly aggregatable species $A\beta_{42(43)}$.

Our epidemiological studies and those of others[9] are supported by genotype-phenotype correlations that suggest that the −48 C/T polymorphism might exert a functional role by influencing *PS1* gene expression[17] and Aβ load, as described here. Other polymorphisms have been reported in the *PS1* promoter, which appear to be in linkage disequilibrium with the −48 C/T polymorphism.[9] Further epidemiological and functional studies are required to determine which of these modifies the risk of AD. These data emphasise the potential importance of control of gene expression in the pathogenesis of AD. Genetic variability in the APP promoter has been suggested to increase the risk of late onset AD,[18] and we have recently reported that polymorphisms in the *APOE* promoter modulate risk for AD.[19 20]

In conclusion, our findings are consistent with the established effects of *PS1* mutations on APP metabolism, suggesting that variations in the level of *PS1* expression per se may have an impact upon AD pathology.

J-CL is a Postdoctoral Marie Curie Fellow. The authors wish to thank Ms M Baba and A Takeuchi for their technical assistance. The study was supported by South Birmingham Mental Health Trust.

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